AD-A136 160 NUCLEAR MAGNETIC RESONANCE RELAXATION AND WATER CONTENTS IN NORMAL TISSUE..(U) PENNSYLVANIA HOSPITAL PHILADELPHIA DEPT OF MOLECULAR BIOLOGY.. 1/1

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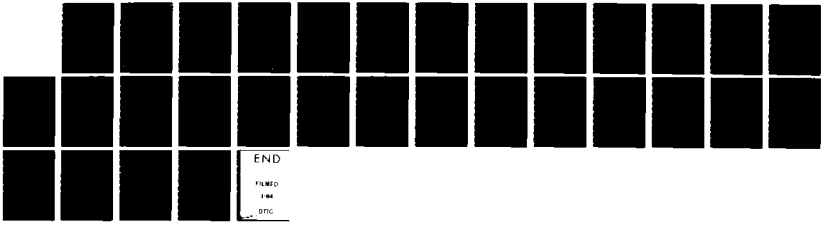

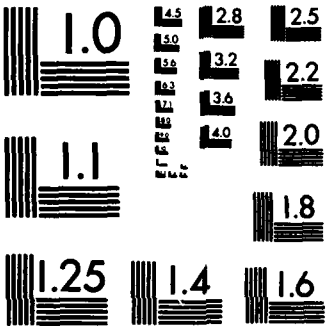

(2)

NUCLEAR MAGNETIC RESONANCE RELAXATION AND WATER CONTENTS IN NORMAL TISSUES AND FIVE TYPES OF CANCER CELLS

AD-A136 180

by

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In 1971 Damadian (1) reported different NMR relaxation times for water protons in 6 normal rat tissues and in 3 malignant tumors. The lower relaxation times T_1 and T_2 in normal tissues were considered by Damadian as consistent with Ling's theory of cell water (2,3,4,5), in which the bulk of cell water exists in the state of polarized multilayers. The slower relaxation seen in cancer tissues was then interpreted by Damadian as indicative of a loosening up of water structure of normal cells as ~~was~~ had been suggested by Szent-Györgyi from a consideration of the differences in the patterns of metabolism between cancer and normal tissues. (6) At that time workers on this subject agreed with Damadian's original interpretation of the cause of the lengthening of the relaxation times of cancer water proton (7,8,9,10). However alternative interpretation soon emerged. By and large, these new considerations were based on the concept that there is rapid exchange of water protons between a major population of water and a minor population (11 to 19). The major population of water protons is assumed to be simply normal liquid water which comprises the bulk of cell water. The minor population of water protons is assumed to be water associated with either paramagnetic ions or with proteins. The minor fraction of water protons have short T_1 and T_2. The observed relaxation times of the living cells are then seen as the weighted averages of the slowly relaxating bulk-phase water and the rapidly relaxing minor-phase water (19). The longer relaxation time of cancer tissue water protons is regarded as due to either a decline in the protein-associated rapidly relaxing water (or "non-freezable" water, 16,17,18) or an altered content of paramagnetic ions (20,21). However a survey of the literature

shows that much of the investigations of paramagnetic-ion effect ended on a negative note (22 to 25).

While most workers readily agree that the addition or removal of water in living tissues or model systems causes water proton relaxation time to lengthen or shorten respectively, several workers have presented evidence showing that the longer relaxation times seen in cancer tissues could not be attributed solely to a larger water content. Thus Beall, Cailleau and Hazlewood (26) as well as Lewa and Baczkowski (27) showed that T_1 of water protons in tissues can vary without a corresponding variation of water contents. Similarly, Kasturi et al (28) and Kagimoto et al (25) could not find positive correlation between water proton relaxation times and water contents they measured. Nevertheless, a substantial number of influential investigators believe that the longer relaxation time of water proton in cancer tissues is simply the result of the higher total water contents (13,14,15,16,17,18,29,30). Indeed both Hollis's group in Baltimore (29) and Pintar's group in Waterloo, Canada (31) were able to plot on a single curve the T_1's against the water contents of various normal, embryonic and cancerous tissues. By treating mouse liver and kidney with varying concentrations of NaCl, thereby causing them to lose or gain water, Eggleston et al (29) were able to show that the H_2O vs. T_1 plots of these two tissues fall on the same line. Inch et al (32) further showed that the water contents vs T_1 plots of various tissues

studied roughly fall between the limits of similar curves of gelatin gel and cotton dispersed in water. These workers all concluded that T_1 is primarily dependent on the total water content of the samples.

The present article, the first of a series, reports experimental efforts aimed at further elucidation of the cause of the differences in T_1 and T_2 between normal and cancer tissues. More specifically, we have attempted to answer the question, "Is the larger cell water content the primary cause of the lengthening of water proton relaxation times in cancer cells?" We chose to study exclusively tumors in the ascites form, which represent cancer cells free of contaminating normal cells.

Materials and Methods

We used in ascites form the following strains of mouse cancer cells. Ehrlich carcinoma carried in ICR mice, and sarcoma 180 were carried in CD-1 mice from Charles River. A methycholanthrene-induced fibrosarcoma (Math A) was carried on female Balb/cJ from Jackson Laboratory. Two strains of rat cancer cells, Novikoff hepatoma, and AS30D hepatoma, the latter of apparently epithilial origin induced by 3' - methyl-4-dimethylazobenzene, were carried in Sprague-Dawley rats from A.R. Schmidt Co., Madison, Wis.

To vary the water contents of slices of normal tissues and cancer cells, they were incubated with gentle shaking of 25°C for 2 hours in one of the five different incubation solutions prepared by mixing normal Krebs solution (NKS) with varying proportions of a hypertonic solution containing, besides all the normal ingredients of NKS, 500 mM of sucrose (NKS + sucrose) or by mixing

NKS with a hypotonic solution, i.e., NKS minus its NaCl (NKS-NaCl). NKS itself contains the following ingredients: NaCl (121.3 mM), KCl (5.0 mM), $NaHCO_3$ (22.5 mM), NaH_2PO_4 (1.2 mM), $MgCl_2$ (1.2 mM), $CaCl_2$ (2.5 mM), glucose (5.6 mM).

After incubation, some normal tissue slices were blotted dry on filter paper, weighed on a torsion balance, and dried at 100°C for the determination of water contents. Other samples were placed in NMR sample tubes for relaxation time studies. Ascites cells were separated from incubation solution by spinning in a Sorvall centrifuge at 40,000 g for 10 minutes in 0.5 ml. microcentrifuge tubes. Part of the ascites cells spun down were weighed fresh and weighed again after drying to obtain their water contents and dry weights; other samples were introduced into the bottom open ends of NMR tubes with both open ends, followed by plugging the bottom with a small teflon button.

Water proton NMR relaxation times T_1 and T_2 were measured, using a coherent CPS-2 NMR pulse spectrometer (Spin Lock, Ltd., Port Credit, Canada) operating at a resonance frequency of 17.1 MHz. T_1 was determined with 180°-τ-90° pulse sequence; T_2 with Carr-Purcell-Meiboom-Gill spin-echo methods. The sample temperature was $25^\circ \pm 1.0^\circ$C.

For studies of water proton spectra of rapidly mixed ascites cells and Co^{++}- or Mn^{++}-doped ascites fluids, the separated cell-free fluid was rapidly mixed with a suitable amount of isotonic $CoCl_2$ or $MnCl_2$. The cells and the Co^{++}- or Mn^{++}-doped fluids were then rapidly mixed and the water proton spectrum read as soon as possible on a continuous wave Jeol JNM-MH-60 spectrometer system.

For studies of ascites cells with varying amount of "extracellular fluids", (ECS) the spundown ascites cells were either suspended in different proportions of normal Kreb's solution to provide cells with high ECS or spun down again for different lengths of time at different centrifugal forces to provide cells with low ECS.

The ECS of the dilute suspensions were obtained from the proportion of the volume of NKS added to the cells. The ECS of the "dense" suspensions were determined with the aid of C^{14} labelled inulin.

Results

Weissman, et al (33,34) first pointed out that larger water contents in neoplastic tumors might have accounted for the longer NMR relaxation times observed. The dispersed nature of the cancer cells permits experimental examination of the effects of changes in chemical composition as well as the effects of the relative amount of the extracellular water on NMR water proton relaxation times of the cancer cell suspensions.

- Rate of exchange of water between Ehrlich ascites cells and its surrounding media.

It has been known that the presence of paramagnetic ions like Co^{++} and Mn^{++} in an aqueous solution produces a two-fold effect on a continuous wave NMR spectrum of water protons: (1) a line broadening and (2) a chemical shift. Dea, et al (35) showed that inclusion of 2 mM Mn^{++} in a Ringer solution bathing rabbit nerve trunks, seperates the water proton signal of the doped extracellular water from the (undoped) intracellular water. Apparently in this case, the rate of exchange of water protons between intra- and extracellular phases is too slow to allow a merging of the two signals. Fig. 1 shows a different picture: When Ehrlich ascites cells were mixed with a Ringer solution containing 50 mM $CoCl_2$, the different water peaks immediately merge into one single broad peak. Doping the Ringer solution with 5 mM $MnCl_2$, and mixing it with Ehrlich ascites cells produced also an instant merging of the two water proton signals.

Thus water protons in these Ehrlich ascites cells in contrast to normal rabbit nerve trunks exchange rapidly with water protons in the external fluids.

Equally important, the data shown in Fig. 1 emphasizes how two populations of water one intracellular and one extracellular can produce, by rapid exchange, a single peak indicating a single relaxation time. Next we shall attempt to find out the NMR water proton relaxation time of pure cancer cells without its being affected by extracellular fluids.

T_1 and T_2 of pure Ehrlich ascites cells.

In Figure 2 we plotted the percentage of extracellular fluid (ecf) of Ehrlich ascites cell suspension against the T_1 and T_2 measured. By extrapolation of the curves to ecf = 0, one obtains the T_1 and T_2 of 800 and 60 milliseconds respectively for the pure ascites cells. From these data one can also conclude that reasonably accurate data of T_1 and T_2 of ascites cells can be simply obtained by spinning 45,000 g for 10 minutes - a condition that produced the group of data marked by the half filled circles in Figure 2 with ECS around 5%.

A comparison of T_1 of normal mouse tissues and of mouse Ehrlich ascites cells.

Table 2 lists our own T_1 and H_2O content data of tissues of ICR mice, the host animal strain for the Ehrlich ascites cells. Our data agree well with both groups of data from Frey et al (10) and from Hollis et al (36). In absolute values, our data agree with Frey et al's data better but are higher than those of Hollis et al. This difference is not due to a difference in the resonance frequency; ours is at a lower frequency (17.1 MHz) than both Frey et al's (30 mMHz) and Hollis et al's (24.3 mHz) and T_1 increases with resonance frequency (see Othred and Genge (12), for T_1 of frog muscles at varying resonance frequencies). Similar data for rat and frog tissues are presented in Tables 3 and 4.

Figures 3 and 4 plot T_1 vs. H_2O contents of normal mouse and rat tissues and of the respective cancer cells. These data suggest that the elevated T_1 value of the cancer cells is not simply due to a large water content. Indeed the water contents of approximately 80% in the cancer cells are equal to or actually lower than some of the normal mouse tissues with much shorter T_1.

T_1, T_2 and water contents of other cancer cells.

Table 5 presents T_1 and T_2 as well as the water contents of three mouse cancers (Ehrlich carcinoma, Sarcoma 180, and Meth. A) and two rat cancers (Novikoff hepatoma,

and AS30D hepatoma) all in ascites form. Considering the diverse origins of these cancer cells, it is remarkable how close their T_1 and T_2 as well as water contents are to each other.

A closer look at the relation between T_1 and water content.

The normal tissue data presented in Figures 3 and 4 show no simple dependence of T_1 on water contents. To investigate this problem further, we varied the tissue water contents by equilibration in hypo- and hypertonic as well as isotonic Ringer solution. We then plotted the T_1 measured against the water contents (Figures 5 and 6). In contrast to the data of Eggleston (29), our experimental points do not fall on a single line. Indeed, each organ presents a distinctly different T_1 at each level of water content. These data strongly argue for the case that T_1 is not simply a function of water contents. Other factors quite specific to each tissue make important contributions toward the T_1 value, although the data also clearly show that alteration of water content unquestionably alters T_1.

In Figures 7 and 8, T_1 and H_2O contents of mouse and rat cancer cells are similarly plotted. In each of these graphs the cross-hatched area covers the distribution of T_1 in normal tissues of the corresponding animal. Thus within the still limited data we have on hand, there is little overlap in the T_1 vs. H_2O content between cancer and normal cells.

Discussion

Of the five strains of ascites tumors, two are hepatomas (Novikoff and AS30D), one is a mammary carcinoma (Ehrlich, ascites), one is a polymorph-cell sarcoma (Sarcoma 180) and another is a fibrosarcoma (Meth. A). In spite of this diverse origin, their remarkably similar T_1's and T_2's water contents contrast sharply with the wide range (600 to 1600 msec) of T_1 observed in human tumors (37), and in solid rat hepatoma tumors (380 msec to 710 msec) (38).

One cause for this difference may be the relatively greater purity of the ascites cancer cells. On the other hand, all solid tumors contain extraneous tissues including blood vessels and their contents, connective tissues, necrotic and dead cells, etc., adding normal liquid water to the system and thereby increasing the apparent NMR water proton relaxation times.

The large collection of tumor water proton T_1 from Damadian et al shows an average T_1 above 1 second. A consultation of Figure 2 shows that if the solid tumor contains say 30 to 40% "extracellular space" water such T_1 value could have been observed.

Let us now compare our data with the interesting study of Hollis, Saryan, Eggleston and Morris (38) in which they showed that the T_1 of their series of hepatomas lie between about 380 msec to 710 msec. The lower range of T_1 in these tumors are quite different from our data. Since Morris and Wagner (39) have long expressed the opinion that their transplantable Morris hepatomas are of the "minimal deviation" type, the constant and consistently high T_1 in our five strains of cancer cells could indicate they are all "maximally deviated" status.

We have already pointed out that the data shown in Figures 3 and 4 do not support the notion that the T_1 differences in living tissues, normal as well as cancerous, are due primarily to their differences in total water contents. However, these data also clearly and unequivocally demonstrate that water content change does affect NMR water proton relaxation times. The next question is, "How much of the observed T_1 difference is due to water content differences?" The data presented permit us to make an estimate.

We chose three normal tissues, kidney, liver, and spleen from mice and rats. Ehrlich carcinoma cells are chosen to represent mouse cancer cells and Novikoff hepatoma to represent rat cancer cells. As shown in Table 6, we first obtained the differences between the T_1 of each normal tissue and its corresponding cancer cells at their

respective normal water contents (Column 4). Next from the data of Figures 5 and 6 we obtained the differences between the T_1 of each normal tissue and tne corresponding cancer cells after normalizing the data to the same water content equal to that of the water content of untreated cancer cells (i.e., 80.8% for mouse tissues and 81.3% for rat tissues)(Column 5).

From these data the percentage of T_1 differences between cancer cells and each normal tissue due to water content (Column 6) and non-water content causes (Column 7) were calculated. These calculations show that water content difference is decidely a minor cause of tne T_1 differences between normal tissue and cancer cells, amounting to an average below 10%.

The elimination of total water content variation as the primary cause of the NMR water proton relaxation times leaves only two other general mechanisms for the observed differences of T_1 between normal tissues and cancer cells: (i) alteration in the contribution of a minor phase water population associated with paramagnetic materials or with proteins, and/or (ii) loosening of dynamic structuring of the bulk phase water normally existing in the state of polarized multilayers.

Summary

By studying NMR water proton spin lattice relaxation times (T_1) of normal mouse and rat tissues at varying water contents and by comparing the data obtained with similar data obtained from 5 strains of pure cancer cells, we reached the conclusion that differences in total water contents between normal tissues and cancer cells contributes less than 10% to the differences between the longer T_1 in cancer cells than in normal tissues.

In spite of the diversity of the origins of the 5 strains of cancer cells studied (all in the ascites form) their T_1 and T_2 as well as water contents are confined to between relatively narrow limits, suggesting the physical state of water in all "maximally deviated" cancer cells is very similar.

In these "pure" cancer cells studied, the spun-down cell pellet contains about 5% extracellular fluid (ecf). However by studying T_1 and T_2 with varying amount of ecf and by extrapolation to zero ecf, we obtained T_1 and T_2 of pure Ehrlich cancer cells without ecf. These values are close to values from cells with 5% ecf. Other studies indicate rapid exchange of water proton between the inside and outside of Ehrlich carcinoma cells.

Acknowledgement

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1968

Legends

Figure 1 - The immediate merging of the water proton signals from Ehrlich carcinoma cells and Co^{+++}-doped ascites fluid. The water proton signal of the Co^{+++}-doped ascites fluid merges with the water proton signal as soon as the cells of the fluid were mixed. The time from mixing to signal recording is less than 1 minute.

Figure 2 - Variation of T_1 and T_2 of water protons from suspensions of Ehrlich carcinoma cells in varying amounts of normal Kreb's solution referred to as extracellular fluid.

Figure 3 - T_1 of normal mouse tissues and T_1 of spun down ascites cells of mouse Ehrlich carcinoma, mouse sarcoma 180, and mouse Meth. A fibrosarcoma plotted against their respective water contents.

Figure 4 - T_1 of normal rat tissues and of spun down ascites cells of rat Novikoff hepatoma and rat AS30D hepatoma.

Figure 5 - T_1 of normal mouse tissues at varying water contents.

Figure 6 - T_1 of normal rat tissues at varying water contents.

Figure 7 - T_1 of 3 strains of mouse cancer cells with varying water contents. Shaded area covers the ranges of T_1 and H_2O contents of normal tissues and is taken from Figure 5.

Figure 8 - T_1 of two strains of rat cancer cells with varying water contents. Shaded area covers the ranges of T_1 and H_2O contents of normal rat tissues and is taken from Figure 6.

Table 1 - Composition of incubation solutions of different osmolarity

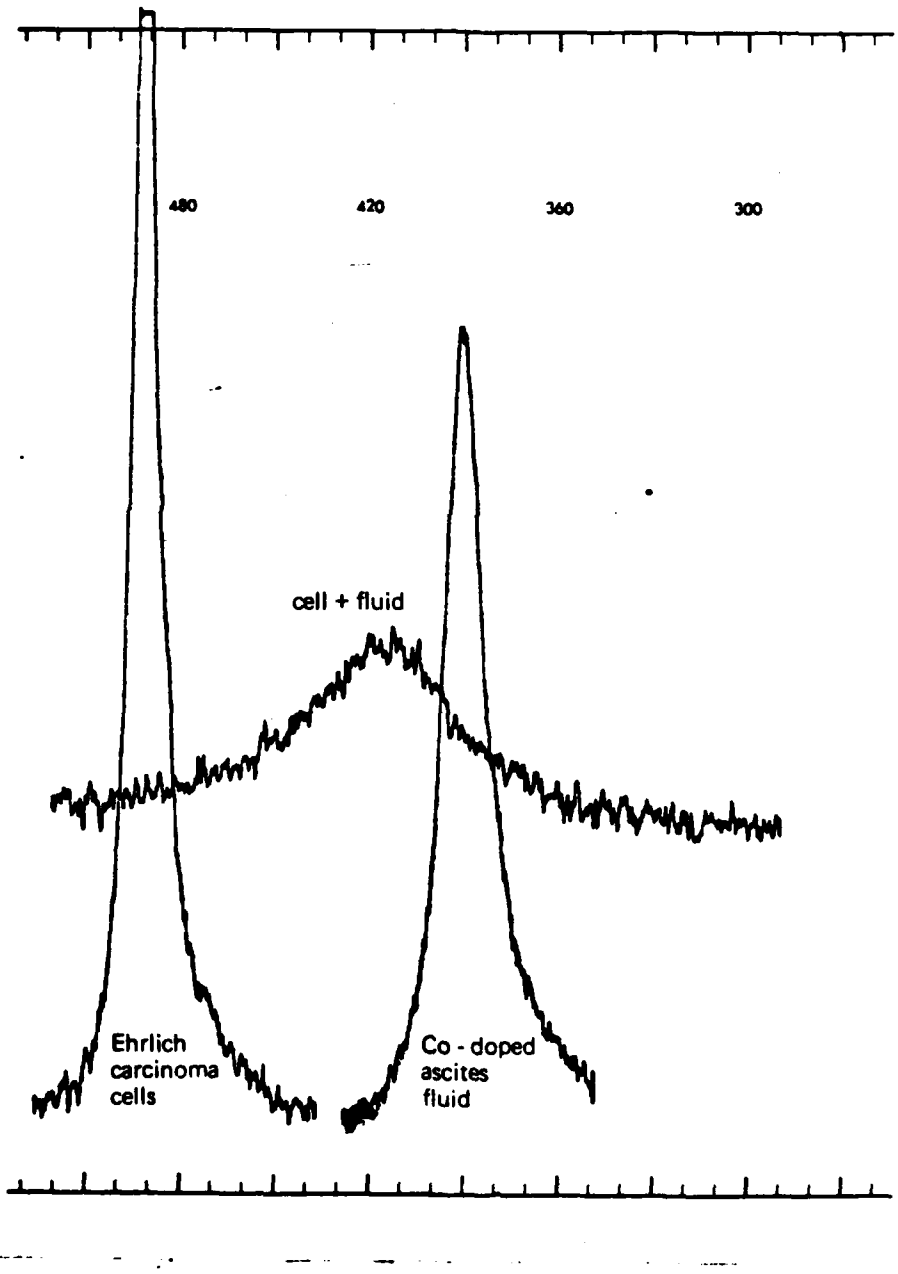
Table 2 - T_1 of H_2O contents of normal mouse tissues.

Table 3 - T_1 and H_2O contents of normal rat tissues

Table 4 - T_1 and H_2O contents of normal frog tissues

Table 5 - T_1 and T_2 of 5 strains of cancer cells.

Table 6 - An extimation of the relative contribution of large water contents in cancer cells to its longer T_1.


480
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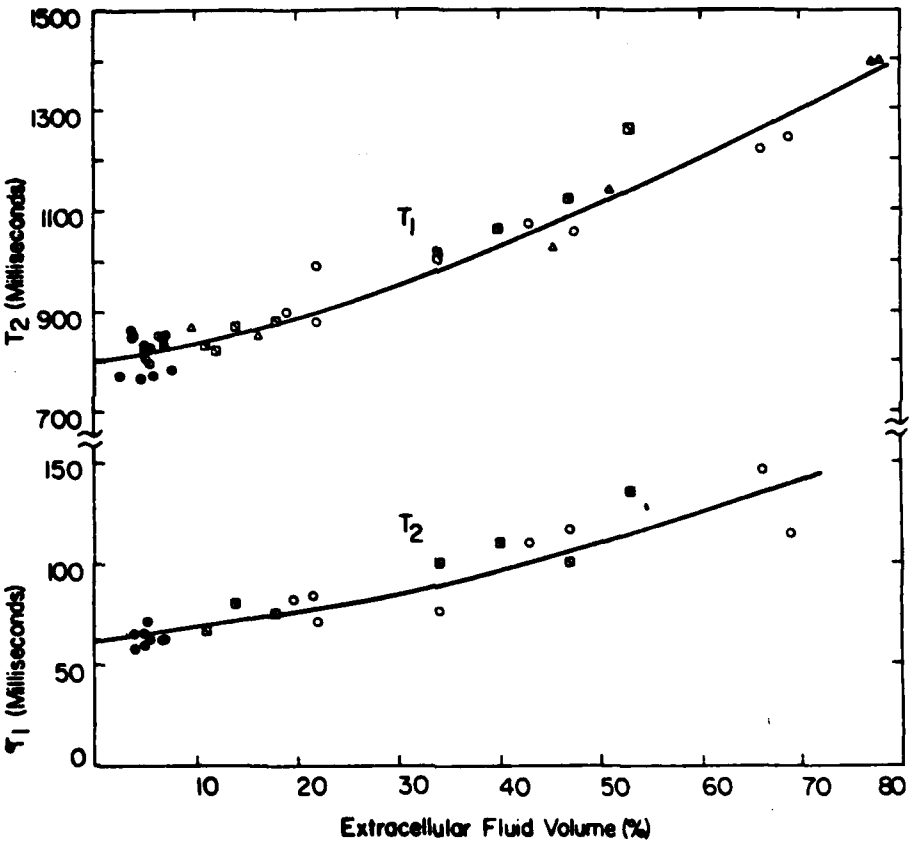
cell + fluid
Ehrlich carcinoma cells
Co - doped ascites fluid

FIGURE 1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1300
1100
900
700
T_2 (Milliseconds)
T_1
150
100
50
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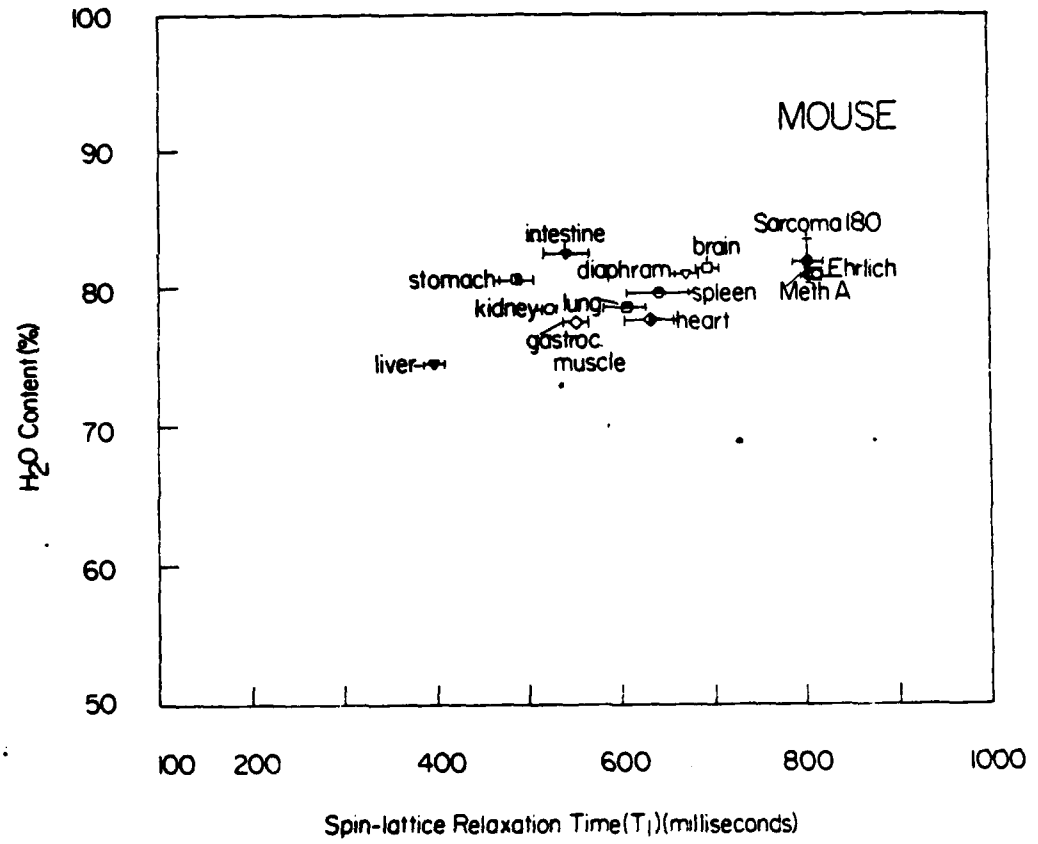
T_1 (Milliseconds)
T_2
10
20
30
40
50
60
70
80
Extracellular Fluid Volume (%)

FIGURE 2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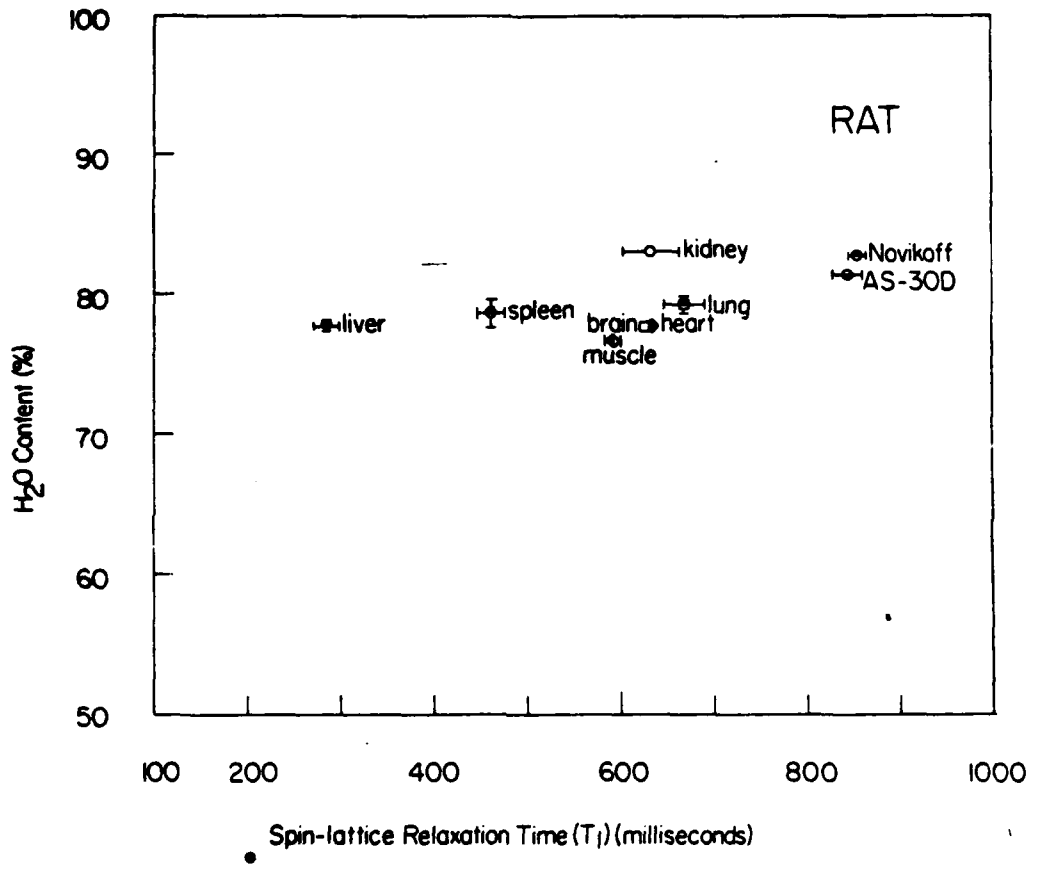
intestine
brain
Sarcoma 180
Ehrlich
stomach
diaphram
spleen
Meth A
kidney
lung
heart
gastroc.
liver
muscle
100
90
80
70
60
50
H_2O Content (%)
100
200
400
600
800
1000
Spin-lattice Relaxation Time(T_1)(milliseconds)

FIGURE 3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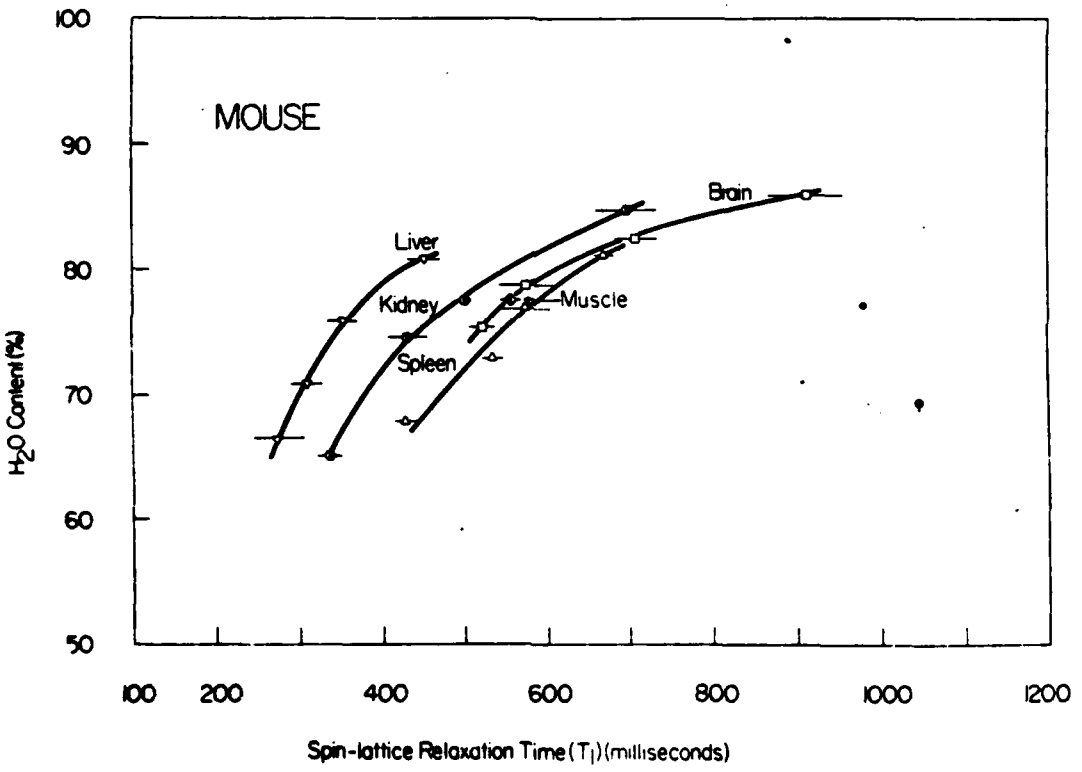
kidney
Novikoff
AS-30D
liver
spleen
lung
brain
heart
muscle
100
90
80
70
60
50
100
200
400
600
800
1000
H2O Content (%)
Spin-lattice Relaxation Time (T1) (milliseconds)

FIGURE 4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Brain
Liver
Kidney
Muscle
Spleen
H_2O Content (%)
100
90
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200
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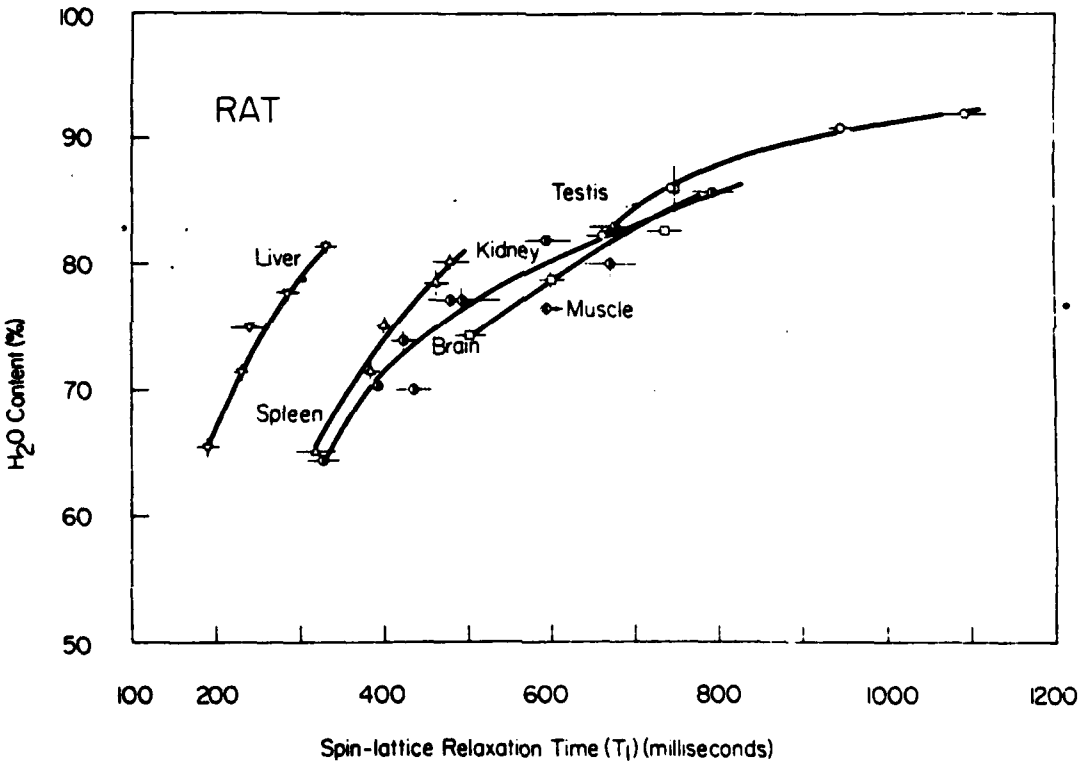
600
800
1000
1200
Spin-lattice Relaxation Time (T_1) (milliseconds)

FIGURE 5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Liver
Kidney
Testis
Muscle
Brain
Spleen
H_2O Content (%)
100
90
80
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400
600
800
1000
1200
Spin-lattice Relaxation Time (T_1) (milliseconds)

FIGURE 6

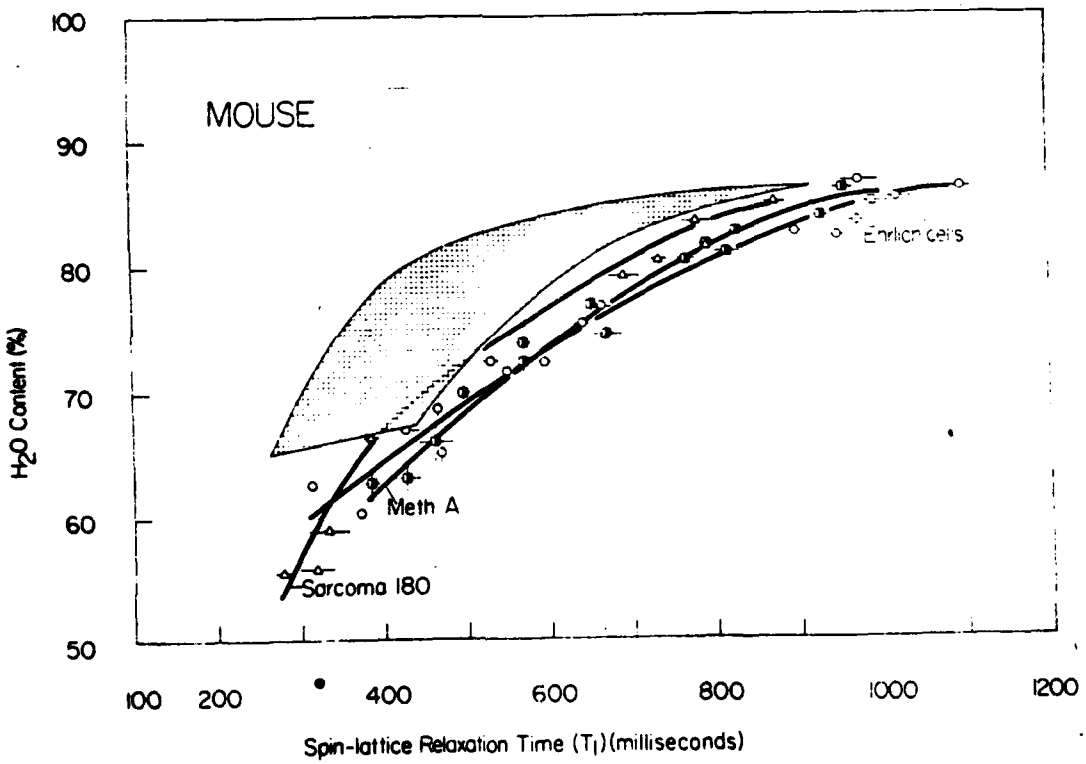
MOUSE
100
90
80
70
60
50
H_2O Content (%)
Ehrlich cells
Meth A
Sarcoma 180
100
200
400
600
800
1000
1200
Spin-lattice Relaxation Time (T_1)(milliseconds)

FIGURE 7

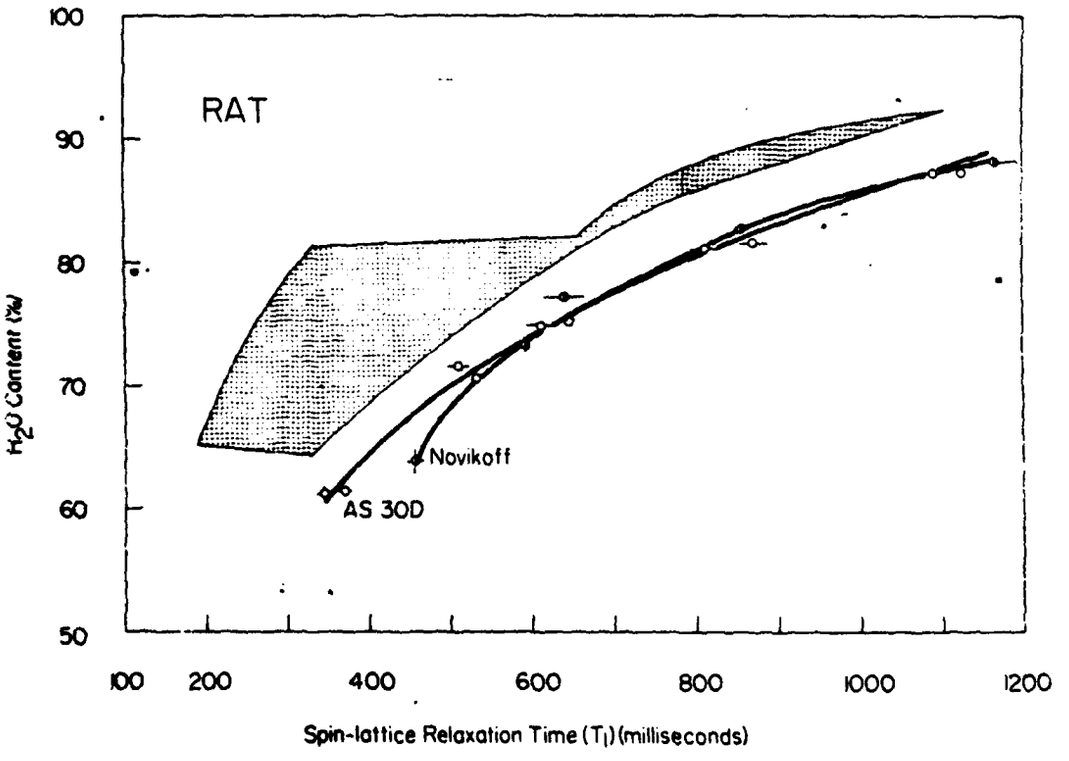
RAT
Novikoff
AS 30D
H_2O Content (%)
100
90
80
70
60
50
100
200
400
600
800
1000
1200
Spin-lattice Relaxation Time (T_1) (milliseconds)

FIGURE 8

TABLE 1

Composition of Incubation Solutions of Different Osmolarity

	Total Osmolarity (mM)	NKS-NaCl	NKS	NKs + sucrose
I	612.5	0	41.2	58.8
II	406.4	0	82.4	17.6
III	318.6	0	100	0
IV	236.4	33.3	66.6	0
V	195.3	50.0	50.0	0
VI	72.0	100	0	0

TABLE 2

Spin-lattice relaxation times of normal mouse tissues

Source	Frey et al (1972)		Hollis, et al (1973)		Ling, et al (to be published)	
Frequency (MHz)	30		24.3		17.1	
Animal Strain	C3H	Swiss	C57B/6J	A/J	ICR	
					T_1	H_2O%
Brain	646	693	526	508	693 ± 13	81.2 ± 0.16
Heart	650	664	491	500	632 ± 30	77.5 ± 0.29
Kidney	470	520	370	314	524 ± 7.5	78.3 ± 0.42
Liver	386	382	263	283	396 ± 12	74.5 ± 0.62
Lung	641	657	490	476	607 ± 27	78.4 ± 0.39
Muscles	615	635	471	415	552 ± 16	77.4 ± 0.23
Spleen	571	563	458	433	641 ± 39	79.5 ± 0.10

TABLE 3

Spin-lattice Relaxation Times of Normal Rat Tissues

Source	Chaughule, et al, 1974	Block and Maxwell, 1974	Bovee, et al, 1974	Ling, et al (to be published)	
Frequency(MHz) -	25.3	13.56	60.0	17.1	
Animal Strain -	C3H jax	Sprague-Dawley	WAG-RY	Sprague-Dawley	
				T_1	H_2O
Brain	472	474		626 ± 3.12	77.6 ± 0.25
Heart	518			633 ± 4.79	77.7 ± 0.15
Kidney	577	410	668	633.8 ± 30.3	77.8 ± 0.63
Liver	273	238	467	285 ± 13.2	77.7 ± 0.35
Lung	587			668 ± 22.1	79.2 ± 0.56
Muscle		404	850	592 ± 7.70	76.6 ± 0.25
Spleen	457		582	461.3 ± 15.2	78.6 ± 0.98

TABLE 4

H_2O Contents and Spin-lattice Relaxation Times of Frog Tissue

	T_1 (msec.)	H_2O Content (%)
Brain	748±30.2	84.9±0.27
Heart	623±15	80.3±0.32
Kidney	370±8.4	77.0±0.33
Liver	281±10.0	68.3±0.36
Muscle	547±38	77.4±0.52
Skin	375±9.2	71.4±1.02
Spleen	577±37.0	75.1±0.40
Stomach	560±7.1	79.7±0.53
Tendon	655±27	78.3±1.31
Testis	592±23	82.1±1.10

TABLE 6

The Relative Contributions of Differences in Their Water Contents and Other Causes of Normal and Cancer Tissues to Their Differences in T_1

		T_1		Total T_1 Difference between normal & cancer tissue (msec.)	T_1 Difference at Equal Water Contents between Normal & cancer tissues (msec.)	Percentage Difference of T_1 due to H_2O Content	Percentage Difference of T_1 Due to Other Causes
		At Normal Water Content (msec.)	At Equal Water Contents (msec.				
Mouse	kidney	524	566	294	252	14%	86%
	liver	396	450	422	368	13%	87%
	spleen	641	650	177	168	5%	95%
	Ehrlich carcinoma	815	815				
Rat	kidney	634	625	221	230	4%	96%
	liver	285	325	570	530	7%	93%
	spleen	461	495	395	360	9%	91%
	Novikoff hepatoma	855	855				
Mean $\pm$ S.E.						8.6%$\pm$1.7%	91.3%$\pm$1.7%

TABLE 5

H_2O Contents, T_1 and T_2 of Cancer Cells

Ascites tumor	Host Animals	H_2O Content (%)	T_1 (msec.)	T_2 (msec.)
Ehrlich carcinoma	mouse	80.8±0.34(n=20)	815±7.07(n=20)	61.6±1.87(n=10)
Sarcoma 180	mouse	81.7±1.6(n=8)	802.5±15.5(n=8)	86.3±3.20(n=8)
Meth. A fibrosarcoma	mouse	80.8±.49(n=8)	805±10.4(n=8)	68.2±1.31(n=8)
Novikoff hepatoma	rat	82.7±.12(n=4)	855±8.66(n=4)	96.9±1.2(n=4)
AS-30D hepatoma	rat	81.3±0.3(n=8)	843.6±16.4(n=8)	80.6±2.13(n=8)

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